Diversity of cultivable endophytic bacteria associated with halophytes in Xinjiang of China and their plant beneficial traits

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Abstract: Endophytic bacteria from halophytes have a wide range of application prospects in various fields, such as plant growth-promoting, biocontrol activity and stress resistance. The current study aimed to identify cultivable endophytic bacteria associated with halophytes grown in the salt-affected soil in Xinjiang Uygur Autonomous Region, China and to evaluate their plant beneficial traits and enzyme-producing activity. Endophytic bacteria were isolated from *Reaumuria soongorica* (PalL Maxim.), *Artemisia carvifolia* (Buch.-Ham. ex Roxb. Hort. Beng.), *Peganum harmala* L. and *Suaeda dendroides* (C. A. Mey. Moq.) by using the cultural-dependent method. Then we classified these bacteria based on the difference between their sequences of 16S rRNA (16S ribosomal RNA) gene. Results showed that the isolated bacteria from *R. soongorica* belonged to the genera *Brucella*, *Bacillus* and *Variovorax*. The bacteria from *A. carvifolia* belonged to the genera *Micromonospora* and *Brucella*. The bacteria from *P. harmala* belonged to the genera *Paramesorhizobium*, *Bacillus* and *Peribacillus*. The bacteria from *S. dendroides* belonged to the genus *Bacillus*. Notably, the genus *Bacillus* was detected in the three above plants, indicating that *Bacillus* is a common taxon of endophytic bacteria in halophytes. And, our results found that about 37.50% of the tested strains showed strong protease-producing activity, 6.25% of the tested strains showed strong cellulase-producing activity and 12.50% of the tested strains showed moderate lipase-producing activity. Besides, all isolated strains were positive for IAA (3-Indoleacetic acid) production, 31.25% of isolated strains exhibited a moderate phosphate solubilization activity and 50.00% of isolated strains exhibited a weak siderophore production activity. Our findings suggest that halophytes are valuable resources for identifying microbes with the ability to increase host plant growth and health in salt-affected soils.

Keywords: endophytes; environmental microbiology; halophytes; biodiversity; plant beneficial properties

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1 Introduction

Xinjiang Uygur Autonomous Region has the biggest land area in China, which accounts for about one-sixth of the total territory (Li et al., 2004). However, most of the lands in Xinjiang are covered by deserts and saline-alkali soils (Huang et al., 2006). Furthermore, with the continuous growth of agriculture and the frequently disturbance of human activities, the problems of soil desertification and salinization are becoming more and more serious, which have aroused a very severe environmental challenge (Hu et al., 2012; Jiang et al., 2020).

Halophytes can survive in the highly saline and arid soils and obtain a high biomass in these soils (Khan et al., 2016). Thus, halophytes have great economic value and ecological significance, such as restoring vegetation in saline soils, improving soil biodiversity and increasing crop productivity (Shamsutdinov et al., 2017).

Endophytes are non-pathogenic microorganisms that live in the tissues and organs of healthy plants at certain or all growth stages, including endophytic bacteria, actinomyces and fungi (Tosi et al., 2021). Endophytic actinomycetes and bacteria belong to the kingdom of bacteria of prokaryotes in taxonomy. But from the morphological point of view, actinomycetes and bacteria are quite different. The cell morphology of actinomycetes is mostly filamentous, while bacteria are spherical and rod-shaped. The colony of actinomycetes is dense and difficult to pick up, while the colony shape of bacteria is mostly round and easy to pick up. Both endophytic actinomycetes and endophytic bacteria are very important bio-resource associated with plants. Most endophytes may promote host plant growth, nutrient acquisition and improve the plant's ability to tolerate abiotic stresses, such as drought, salinity and decrease stresses (Farahat, 2020). And, there were pieces of evidence indicating that coevolution happens between endophytes and halophytes (Khare et al., 2018; Taulé et al., 2021). Host plants can provide a suitable living environment and abundant nutrients for endophytes, while endophytes can promote host plant growth by fixing nitrogen, releasing auxin and other secondary metabolites (Wani et al., 2015; Afzal et al., 2019).

Recently, there have been several records of endophytic bacteria associated with halophytes. In previous studies, it had been found Tenericutes, Proteobacteria, Firmicutes and Actinobacteria were mainly endophytic bacteria communities of the sixteen halophytes in Northern Xinjiang by the next-generation sequencing technology (Zhao et al., 2016a). Zhao et al. (2016b) found that the Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria were the main endophytic bacteria communities in *Salicornia europaea* L. roots based on high-throughput sequencing analysis. In addition to having a high diversity, halophyte endophytic bacteria also can promote the growth of their host plants. Teng et al. (2010) isolated four ACC (1-aminocyclopropane-1-carboxylate) deaminase-including endophytic bacterial strains from *Suaeda glauca* (Bunge) Bunge, which were identified as *Pseudomonas* sp., *Pseudomonas oryzihabitans*, *Pseudomonas putida* and *Pantoea agglomerans*. Six isolates resulting in a large rise in rice production were screened out from fifty-nine endophytic bacterial isolates by Khan et al. (2020). Five isolates associated with *S. europaea* displayed significant stimulation of the host plant's growth, which were recognized as *Variovorax paradoxus*, *Bacillus endophyticus*, *Bacillus tequilensis*, *Arthrobacter agilis* and *Planococcus rifietoensis* (Zhao et al., 2016c). Twenty-nine endophytic bacterial strains were isolated from *Prosopis strombulifera* and these endophytic bacterial strains were capable of producing phytohormone, in which six tested isolates were positive for the ACC deaminase activity and one strain had the ability to produce siderophores (Sgroy et al., 2009). However, all isolates couldn't solubilize phosphate. There are many kinds of halophytes in arid areas all over the world. But the community composition and ecological role of endophytic bacteria associated with these halophytes are still not very clear (Zhao et al., 2013).

In this research, we studied the community's composition of cultivable endophytic bacteria associated with four halophytes collected from the salt-affected soils in Xinjiang, China, as well as their plant beneficial traits. The aims of this study are (1) to isolate endophytic bacteria from four halophytes grown in the saline-alkali soils; (2) to recognize the isolated endophytic bacteria via 16S rRNA gene sequencing; and (3) to examine their plant beneficial traits.

2 Materials and methods

2.1 Plant samples collection and pretreatment

Four halophytes, i.e., *R. soongorica* (Tamaricaceae), *A. carvifolia* (Asteraceae), *P. harmala* (Nitrariaceae) and *S. dendroides* (Amaranhaceae) were collected from the saline-alkali soils in the Fukang City (44°13′39″N, 87°40′16″E) of Xinjiang, China. These four plants were named as P1, P2, P3 and P4, respectively in this study.

Four plants were collected from their natural habitats, put in sterile sampling bags, and then transported to laboratory. Each plant sample was flushed in running water to dislodge the soil and mud on the root surface and dust on the plant surface. The plant samples were surface-sterilized by soaking them sequentially in 75% medical alcohol for 5 min and subsequently with 5% NaClO for 8 min and were washed three times using the sterile distilled water (Liu et al., 2017). To verify the efficiency of plant sample surface sterilization, we spread 100 μL the last rinse water onto the Tryptic Soy Agar plates (TSA) and International Strptomyces Project Medium agar plates (ISP2). If there was no colony growth, the plant sample surface sterilization was considered as effective after 3 d of incubation at 30°C (Rajivgandhi et al., 2018; Ramachandran et al., 2019). The surface sterile plants were cut into 1–2 cm pieces with a sterile scalpel and dried in the horizontal flow clean bench. Finally, all of the samples were crushed by a sterile masher and stored at –20°C.

2.2 Isolation of endophytic bacterial strains

One gram of plant sample was ground thoroughly using a sterile mortar and poured into 50 mL centrifuge tube with 9 mL of sterile distilled water and a few sterile glass beads. The sample was shaken at 100 r/min for 30 min using a Shaken Incubator (Shanghai Tensuc Lab Instruments Manufacturing Co., Ltd., China). The supernatant was extracted and transferred into a new sterile centrifuge tube after the tissue homogenate was centrifuged at 3000 r/min for 10 min. Series gradient dilutions (10^{-2}–10^{-4}) were performed by sterile operation, then 70 μL dilutions were spread onto tap water-yeast extract agar (TWYE) medium and Glycerol-Asparagine medium. All plates used to isolate endophytic bacteria were incubated at 30°C for 30 d. The component of TWYE medium was as follows: 0.25 g/L yeast extract, 0.5 g/L K_2HPO_4, 15 g/L agar and pH 7.2 (Wang, 2017). The content of Glycerol-Asparagine medium was as follows: 1 g/L asparagine, 10 g/L glycerol, 30 g/L NaCl, 1 g/L K_2HPO_4, 0.001 g/L $FeSO_4$•$7H_2O$, 0.001 g/L $MnSO_4$•H_2O, 0.001 g/L $ZnSO_4$•$7H_2O$, 15 g/L agar and pH 7.2 (Liao, 1997). After 30 d of culture, colonies with different shapes and colors were picked carefully, transferred onto TSA plates, and then incubated at 30°C for 7 d to check the purity of the isolated endophytic bacteria. Colonies with similar phenotypes (such as sizes, shapes and colors) were duplicated to cut down the number of bacteria to be sequenced and shrink the scope of screening. The isolated strains were preserved with 20% glycerin tube and stored in –80°C.

2.3 Deoxyribonucleic acid (DNA) extraction

The genomic DNA of isolates was extracted using Chelex® 100 sodium. A single colony was transferred into the sterile polymerase chain reaction (PCR) tube with 50 μL of 5% chelex-100 and was mixed thoroughly with a vortex mixer. The tube was incubated for 25 min at 99°C in the PCR instrument (C1000 Touch™ Thermal Cycler, BioRad Laboratories, USA) and then centrifuged at 12,000 r/min for 10 min. The extracted genomic DNA was dissolved in the supernatant and used as the template for the PCR.

2.4 PCR and agarose gel electrophoresis

The amplification of 16S rRNA gene of isolates was performed by using the following universal primers: 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GAAAGGAGGTGATCCAG CC-3') (Biomed, China). The 16s rRNA gene was amplified in a C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories, USA) in a total volume of 50 μL of reaction mixture consisting of 1 μL template DNA, 25 μL 2×Taq MasterMix (Coolaber, China), 1 μL 27F, 1 μL 1492R and 22 μL ddH_2O (Tiangen Biotech Co. Ltd., Beijing, China). PCR was carried out under the following

procedures: pre-denaturation at 94°C for 6 min, followed by 35 cycles of denaturation for 30 s at 94°C, annealing at 55°C for 30 s and extension at 72°C for 1.5 min, followed by the final step at 72°C for 7 min. PCR products with a length of about 1500 bp were checked by 1% agarose gel electrophoresis.

2.5 Sequencing and analysis of phylogenetic tree construction

The positive PCR products were purified and sequenced by Sangon Biotech in Shanghai, China. The received paired-end raw data were merged using the Molecular Evolutionary Genetics Analysis 7.0 (MEGA 7.0) and then were compared with strains registered in the EzBioCloud database utilizing 16S-based identification system. We multiply aligned the sequences of isolates by sequencing and sequences of the most similar strains downloading from EzBioCloud based on the ClustalW method and constructed the evolutionary tree by the Neighbor-Joining method using MEGA 7.0.

2.6 Accession numbers

All endophytic bacterial 16S rRNA gene sequences were stored into the GenBank database following the below accession numbers: MW228433–MW228448.

2.7 Plant beneficial traits

The producing assay of IAA was performed by color reaction method (Amaresan et al., 2012). All strains were inoculated in nutrient broth medium under shaking conditions (120 r/min) at 30°C for 5 d. The culture supernatant was acquired after centrifuging at 5000 r/min for 10 min. After that, 4 mL of Salkowski reagent was added to 2 mL of supernatant and incubated at 30°C for 30 min in a dark room. The color of the mixture changed to pink or red indicating that IAA was produced by the tested strain.

Phosphate solubilization activity was measured by inoculating strains on Pikovskaya medium, then incubated at 30°C for 5 d (Simarmata et al., 2020). The content of Pikovskaya medium was as follows: 0.5 g/L yeast extract, 10 g/L glucose, 5 g/L $Ca_3(PO_4)_2$, 0.5 g/L $(NH_4)_2SO_4$, 0.2 g/L NaCl, 0.2 g/L KCl, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.025 g/L bromophenol blue, 0.002 g/L $FeSO_4 \cdot 7H_2O$, 20 g/L agar and pH 7.0. The presence of a transparent zone around the colony means that the strain will solubilize phosphate.

Siderophore-producing activity was carried out for inoculating strains on Chrome Azurol S agar plate (CAS) and cultivated at 30°C for 5 d. An orange or purple halo zone around the colony comes up and the blue color of the CAS medium changes indicating a positive strain screened for the siderophore production (Khan et al., 2020).

Protease activity was determined by growing the isolates on Skim Milk Agar medium. The positive findings were shown by the presence of a transparent halo region around the bacterial colony after 3 d of incubation at 30°C (Simarmata et al., 2020).

Cellulase activity was tested by inoculating bacterial isolates onto a medium containing a unique cellulase substrate namely carboxy methyl cellulose sodium salt (CMC-Na). After 7 d of cultivation at 30°C, all plates were stained with 5 mL 0.1% Congo red solution for 10 min and then rinsed by using 5 mL 1 M NaCl. The existence of a transparent or softly colored region around the colony signaled the presence of positive endophytic bacterial strains (Simarmata et al., 2020).

The assay of lipase activity was carried out by using bacterial isolates being scratched on Tween80 medium. The content of Tween80 medium was as follows: 10 mL/L Tween80, 50 ml/L Victoria blue-suspension, 10 g/L peptone, 5 g/L NaCl, 0.1 g/L $CaCl_2 \cdot 7H_2O$ and 15 g/L agar. Bacterial isolates were cultivated for 7 d at 30°C. The formation of a clear halo indicated that the result is positive (Mesa et al., 2015).

The enzyme-producing activity was calculated by the formula (three replicates to get the average enzyme-producing activity) (Liu et al., 2017).

$$E = \frac{D1}{D2} \times 100\% , \quad (1)$$

where E is the enzyme-producing activity of endophytic bacteria; $D1$ is the diameter of the clear

zone (mm); and *D*2 is the diameter of the endophytic bacterial isolate (mm).

2.8 Data processing and visualization

The processing and visualization of data are completed by Microsoft Excel 2019, MEGA 7.0 and GENESCLOUD (https://www.genescloud.cn/). Microsoft Excel 2019 was used to complete the data processing and draw pie charts, MEGA 7.0 was used to construct the phylogenetic tree, and GENESCLOUD was used to complete the drawing of the Venn chart.

3 Results

3.1 Isolation and identification of endophytic bacterial strains

A total of sixteen endophytic bacterial strains belonging to three phyla, four classes, four orders, four families and six genera were isolated from four halophytic plants. Table 1 shows the 16S rRNA gene sequence similarities of endophytic bacterial strains isolated from four halophytes with the most related sequences from the EzBioCloud database. All of the isolated independent strains shared 98%–100% of their 16S rRNA gene similarity with those in the EzBioCloud database. The isolation results showed that the predominant genera were *Bacillus*, *Brucella* and *Phyllobacterium*, accounting for 38%, 25% and 19% of the total number of isolates, respectively (Fig. 1). Five bacterial isolates (P1.1–1.5) were isolated from P1, three bacterial (P2.1–2.3) from P2, five bacterial (P3.1–3.5) from P3, and three bacterial (P4.1–4.3) from P4. However, we failed to isolate the endophytic bacteria common to four halophytes according to the Venn diagram of Figure 1b. Three phyla were described among the isolated strains: Actinobacteria (P2.1), Proteobacteria (P1.3–P1.5, P2.2, P2.3, P3.2, P3.3 and P3.5), and Firmicutes (P1.1, P1.2, P3.1, P3.4 and P4.1–P4.3). The isolates were identified as *Bacillus atrophaeus* (P1.2, P4.2 and P4.3), *Bacillus filamentosus* (P3.1 and P4.1), *Bacillus halotolerans* (P1.1), *Brevibacterium frigoritolerans* (P3.4), *Brucella endophytica* (P1.3, P1.5 P2.2 and P2.3), *Micromonospora citrea* (P2.1), *Phyllobacterium phragmitis* (P3.2, P3.3 and P3.5) and *Variovorax paradoxus* (P1.4) (Table 1; Fig. 2). Although there are eight isolates (P1.3–P1.5, P2.2, P2.3, P3.2, P3.3 and P3.5) from the phylum Proteobacteria, they are from different classes, i.e., Alphaproteobacteria (P1.3, P1.5, P2.2, P2.3, P3.2, P3.3 and P3.5) and Betaproteobacteria (P1.4; Table 1). Six isolates of the phylum Firmicutes belong to the genus *Bacillus* and only one isolate of the phylum Firmicutes belongs to the genus *Peribacillus* (Table 1; Fig. 1). The only one actinomycete strain (P2.1) isolated from P2 belongs to the genus *Micromonospora* (Table 1; Fig. 2). Firmicutes (four species) had more isolates than Proteobacteria (three species) and Actinobacteria (one species) at the species level in four halophytes, according to the effect of endophytic bacterial strain isolation (Table 1; Fig. 2). The phylogenetic tree that was constructed revealed the closest species of isolated endophytic bacterial

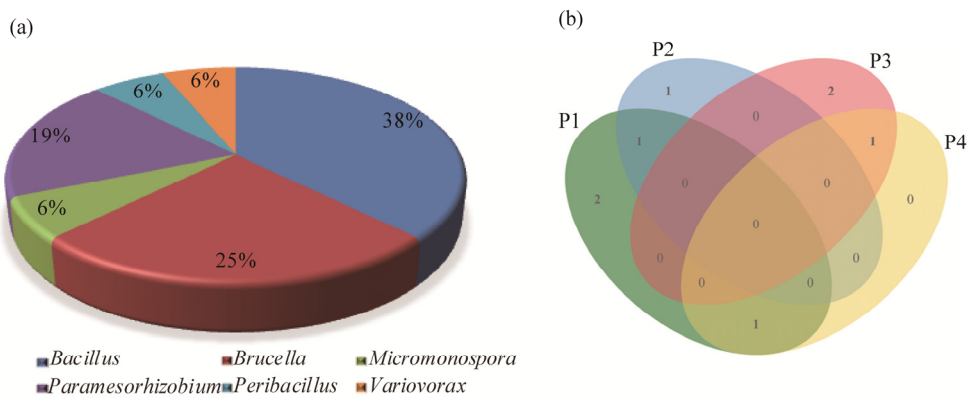

Fig. 1 (a), percentage of isolated strains at the genus level; (b), Venn diagram of endophytic bacteria from four halophytes at the species level. P1, *R. soongorica* (Tamaricaceae); P2, *A. carvifolia* (Asteraceae); P3, *P. harmala* (Nitrariaceae); P4, *S. dendroides* (Amaranhaceae). The abbreviations are the same in the following figures and tables. The numbers in the Venn diagram represent the number of endophytic bacterial species.

strains in the EzBioCloud database (Fig. 2). According to species diversity, the representatives of Firmicutes phyla (4 species) were the highest as compared with Proteobacteria (3 isolates) and Actinomycetes (1 isolate) in halophytes (Fig. 2).

Table 1 Sequence similarities of isolated endophytic bacterial 16S rRNA gene with sequences registered in the EzBioCloud database

Isolated strain			Species with the highest similarity in the EzBioCloud database		
Strain	Size (bp)	Accession number	Top-hit taxon	Identity (%)	Accession number
P1.1	1489	MW228433	*Bacillus halotolerans*	99.45	LPVF01000003
P1.2	1488	MW228434	*Bacillus atrophaeus*	99.38	AB021181
P1.3	1417	MW228435	*Brucella endophytica*	98.35	KP721485
P1.4	1476	MW228436	*Variovorax paradoxus*	99.38	BCUT01000013
P1.5	1427	MW228437	*Brucella endophytica*	98.43	KP721485
P2.1	1450	MW228438	*Micromonospora citrea*	99.09	FMHZ01000002
P2.2	1417	MW228439	*Brucella endophytica*	98.71	KP721485
P2.3	1421	MW228440	*Brucella endophytica*	99.43	KP721485
P3.1	1488	MW228441	*Bacillus filamentosus*	100.00	KF265351
P3.2	1427	MW228442	*Phyllobacterium phragmitis*	98.36	PVBR01000053
P3.3	1424	MW228443	*Phyllobacterium phragmitis*	98.00	PVBR01000053
P3.4	1486	MW228444	*Brevibacterium frigoritolerans*	99.25	AM747813
P3.5	1426	MW228445	*Phyllobacterium phragmitis*	98.50	PVBR01000053
P4.1	1484	MW228446	*Bacillus filamentosus*	99.57	KF265351
P4.2	1483	MW228447	*Bacillus atrophaeus*	99.39	AB021181
P4.3	1485	MW228448	*Bacillus atrophaeus*	99.52	AB021181

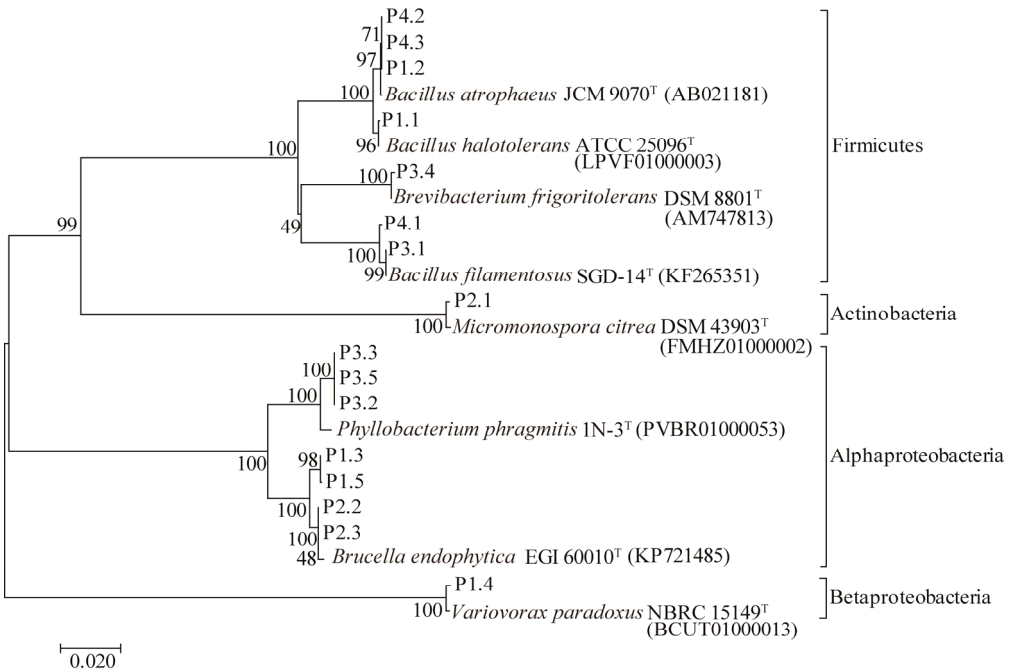

Fig. 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of endophytic bacteria

3.2 Plant beneficial traits

In this study, enzyme-producing activities of sixteen endophytic bacterial strains from four halophytes were screened. Among the tested strains, the highest protease activity was observed in bacterial isolates P1.1, P1.2, P3.3, P3.4, P4.2 and P4.3, while other isolates exhibited moderate or negative protease activity (Table 2). Strong cellulase-producing activity was observed in the P1.1 isolate, and about 31.25% of the tested strains showed negative cellulase activity (Table 2). About 18.75% strains with lipase activity were screened out from all tested endophytic bacteria (Table 2). Except for the enzyme-producing activities, we also screened other plant beneficial traits of endophytic bacterial strains isolated from four halophytes. The findings revealed that all of the test isolates produced IAA. Compared with other strains, the isolated strain P4.3 showed higher IAA production activity (Table 2). And, the isolates P1.4, P2.3, P3.1, P3.2 and P4.2 showed moderate phosphate solubilization activity. The isolates P1.1, P1.2, P2.2, P3.3, P3.4 and P4.3 showed weak phosphate solubilization activity. However, the isolates P1.3, P1.5, P2.1, P3.5 and P4.1 showed negative phosphate solubilization activity (Table 2). Furthermore, half of all isolated strains were able to produce siderophore weakly (Table 2). Through comparative analysis, we found that strain P1.1 had the activity of producing three tested enzymes (Table 2). Moreover, the isolated strains P1.2, P1.4, P2.3, P3.2, P3.3 and P3.4 have the abilities of producing IAA, siderophore and phosphate solubilization (Table 2). Therefore, our findings displayed that these strains have the potential to promote the growth of host plants.

Table 2 Plant beneficial traits of endophytic bacterial strains associated with four halophytes

Isolated strain	Enzyme-producing activity			Plant growth-promoting traits		
	Protease[b]	Cellulase[b]	Lipase[b]	IAA[a]	Phosphate solubilization[b]	Siderophore[b]
P1.1	+++	+++	+	+	+	-
P1.2	+++	-	++	+	+	+
P1.3	-	+	-	+	-	+
P1.4	-	++	-	+	++	+
P1.5	-	++	-	+	-	-
P2.1	++	+	-	+	-	-
P2.2	++	++	-	+	+	-
P2.3	++	+	-	+	++	+
P3.1	-	+	-	+	++	-
P3.2	++	++	-	+	++	+
P3.3	+++	-	-	+	+	+
P3.4	+++	-	-	+	+	+
P3.5	-	++	-	+	-	+
P4.1	-	+	-	+	-	-
P4.2	+++	-	-	+	++	-
P4.3	+++	-	++	++	+	-

Note: IAA, 3-Indoleacetic acid. [a], results are expressed by positive (+) or negative (-) activity of color reaction. ++, moderate activity (red); +, weak activity (pink); -, negative activity. [b], results are expressed by presence (+) or absence (-) of halo. +++, strong activity; ++, moderate activity; + weak activity; -, negative activity.

In the screening results of protease-producing strains, 37.50% of the tested strains had strong protease activity, and 25.00% of the tested strains exhibited moderate protease activity. However, the remaining 37.50% of the tested strains had no performance of protease activity (Fig. 3a). In the screening results of lipase-producing strains, 12.50% of the tested strains exhibited moderate lipase activity, 6.25% of the tested strains exhibited weak lipase activity, and 81.25% of the tested strains had no lipase activity (Fig. 3b). In the screening results of cellulase-producing strains, 6.25% of the tested strains exhibited strong cellulase activity, 31.25% of the tested strains exhibited moderate cellulase activity, 31.25% of the tested strains exhibited weak cellulase activity, and 31.25% of the tested strains had no cellulase activity

(Fig. 3c). In the screening results of IAA-producing strains, 6.25% of the tested strains possessed moderate IAA-producing activity and 93.75% of the tested strains possessed weak IAA-producing activity (Fig. 3d). In the screening results of phosphate solubilization strains, 31.25% of the tested strains possessed moderate phosphate solubilization ability, 37.50% of the tested strains showed weak phosphate solubilization ability, and 31.25% of the tested strains had no phosphate solubilization ability (Fig. 3e). In the screening results of siderophore-producing strains, 50.00% of the tested strains displayed weak siderophore-producing activity and 50.00% of the tested strains did not display siderophore-producing activity (Fig. 3f).

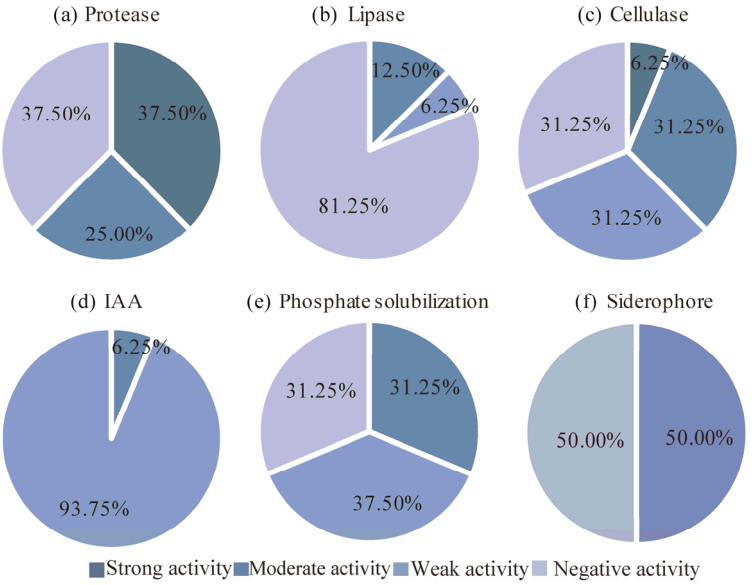

Fig. 3 Proportion of strains with different enzyme-producing activities (a–c) and plant growth-promoting traits (d–f)

4 Discussion

As a result of global climate change, soil salinization has become one of the most serious abiotic stresses impacting plant growth and crop production around the world (Nakbanpote et al., 2014). Plant-associated endophytic bacteria live in plant tissues during their entire lives and are considered to be beneficial to host plant health and growth (Mishra et al., 2017; Fouda et al., 2021). Endophytic bacteria create metabolites that are analogous to those released by the host plant, making them a rich source of secondary metabolites (Gouda et al., 2016). To better understand the community structure and ecological role of endophytic bacteria associated with halophytes, four typical halophytes were chosen for endophytic bacteria isolation and functional strain screening.

At present, there are few studies on endophytic bacteria associated with *R. soongorica*, *A. carvifolia* and *S. dendroides*. In this study, the culture-dependent approach was used to isolate endophytic bacterial strains associated with four halophytes. After identification, we found that seven strains among the sixteen isolates were from the Firmicutes phylum. Strain P1.1 was identified as *Bacillus halotolerans*, strain P3.4 as *Brevibacterium frigoritolerans*, strain P4.1 as *Bacillus endophyticus*, strain P3.1 and P4.1 as *Bacillus filimentosus*, and strains P1.2, P4.2 and P4.3 as *Bacillus atrophaeus* (Table 1). We have also isolated a strain of endophytic actinomycetes identified as *Micromonospora citrea*, which belongs to the *Micromonospora* genus (Table 1). The phylum Proteobacteria contained eight bacterial isolates, belonging to genera *Brucella*, *Paramesorhizobium* and *Variovorax*. Strain P1.4 was identified as *Variovorax paradoxus*, strains P3.2, P3.3 and P3.5 as *Phyllobacterium phragmitis*, and strains P1.3, P1.5, P2.2 and P2.3 as *Brucella endophytica* (Table 1). Lei et al. (2020) isolated a novel Gram-positive, aerobic and motile endophytic actinomycete proposed as *Actinokineospora pegani* sp. nov. from the root of *Peganum harmala*.

In our findings, the endophytic bacterium *Bacillus halotolerans* (P1.1) has capacity for the production of protease, cellulase, lipase, IAA and phosphate solubilization (Table 2). This study on the inoculation of *Bacillus halotolerans* showed that bacterial inoculation led to significant increases in plant development, nutritional content and food quality even under saline stress conditions (Jiménez-Gómez et al., 2021). The taxon we isolated also showed some plant growth-promoting traits, such as IAA production and phosphate solubilization ability (Table 2). The isolated endophytic bacteria *Bacillus atrophaeus* (P1.2 and P4.3) can produce protease and lipase, which is consistent with the finding of Mohamad et al. (2018). In addition, our findings have also revealed that the isolated endophytic bacteria *Bacillus atrophaeus* (P1.2 and P4.3) possessed plant growth-promoting potential (Table 2).

In the present study, the strains P1.3, P1.5, P2.2 and P2.3 were identified as *Brucella endophytica*. It is worth noting that the basonym of *Brucella endophytica* is *Ochrobactrum endophyticum*. In a similar investigation, an endophytic bacterium isolated from the roots of *Glycyrrhiza uralensis* F. was proposed as *O. endophyticum* (Li et al., 2016). However, there are few studies on the plant growth-promoting potential and enzyme-producing activity of *B. endophytica*. According to our findings, *B. endophytica* has certain characteristics of plant beneficial trait and cellulase production (Table 2). The endophytic bacterium P1.4 was isolated from *R. soongorica*, which was identified as *Variovorax paradoxus*, and had the ability to produce IAA, siderophore and phosphate solubilization. The results indicated that the strain P1.4 could promote host plant growth in certain conditions (Tables 1 and 2). Chen et al. (2013) had proved that *V. paradoxus* containing ACC deaminase can promote the growth of *Arabidopsis thaliana*.

We isolated an endophytic strain P2.1, which was identified as *Micromonospora citrea* and belonged to phylum Actinobacteria. It was found that a new family of antibacterial antibiotics could be isolated from *M. citrea* (Carter et al., 1990). Nevertheless, there is no report about plant beneficial traits of *M. citrea*. Our results show that *M. citrea* can produce protease, cellulase and IAA (Table 2).

In our results, there are three endophytic strains with plant beneficial traits isolated from *P. harmala* (P3) growing in the saline soils and identified as *Phyllobacterium phragmitis*. The Gram-negative bacteria were isolated from the *Phragmites australis* rhizome growing in the Kumtag Desert and were first proposed as *P. phragmitis* (Liang et al., 2019). At the moment, there are no relevant references about the plant beneficial traits of *P. phragmitis*. We found that *P. phragmitis* can produce IAA and siderophore, which has the potential to promote plant growth (Table 2). We isolated endophytic bacteria strain P3.4 identified as *Brevibacterium frigoritolerans*, which showed plant growth-promoting traits to some extent and strong protease activity. Unnisa et al. (2021) also found that *B. frigoritolerans* has good plant growth promotion potential such as phosphate solubilization, IAA and ammonia production, and bio-control properties such as hydrogen cyanide production, siderophore production and lytic enzymes production. In our research, the endophytic strains P3.1 and P4.1 were identified as *Bacillus filamentosus* with cellulase activity and capability of IAA production (Table 2). Yahaghi et al. (2019) found that *B. filamentosus* was the most effective in the root and shoot growth stimulation of alfalfa seedlings. To our knowledge, it is rarely reported that strain *B. filamentosus* is isolated from plants, especially halophytes. Besides, it was reported that *B. filamentosus* was isolated from a marine sediment sample for the first time (Sonalkar et al., 2015).

5 Conclusions

A total of sixteen endophytic bacteria associated with halophytes belong to the genera *Bacillus*, *Micromonospora*, *Brucella*, *Paramesorhizobium*, *Peribacillus* and *Variovorax*. All isolated endophytic bacteria showed the ability of IAA production and some isolates were positive for the production of three tested enzymes, siderophores and phosphates solubilization. And these endophytic bacteria play important roles in promoting the growth of host plants and improving their stress resistance. Our research findings also indicate that halophyte is a resource bank for functional microbes that can sustain plant health and growth under harsh climates. Therefore, it is of great economic value and ecological significance to apply functional endophytes to agricultural

production. Furthermore, our findings reveal that further studies in the lab and field experiments are needed to confirm plant growth-promoting traits of endophytic bacteria isolated from halophytes.

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